

Modeling Asthma in Mice What Have We Learned about the Airway Epithelium?

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Clinical reports of areas of damaged airway epithelium associated with shed epithelial cells in bronchoalveolar lavage fluid, aberrant epithelial repair processes, and altered cytokine and growth factor release have highlighted some fundamental differences between the airway epithelium in individuals with and without asthma. However, the consequences of these epithelial changes are not clearly defined, and may be difficult to assess in the clinic. In this Review, we answer the two questions. (1) What *in vivo* models and methods have been used to inform us about airway epithelium damage, repair, and immune responses? Our response focuses on genetic influences as well as allergen exposure, environmental/chemical, and mechanical models. (2) How can we improve on existing mouse models to understand changes in airway epithelium biology in asthma? In answering the second question, we include exciting recent studies that have combined multiple exposure methods and/or epithelium-centric outcome measurements. By addressing these two questions, we propose that future interrogation of epithelial responses of both existing and nascent mouse models may provide greater understanding of the mechanisms underlying airway inflammation and remodeling in asthma with hope of generating novel therapeutic targets.

Keywords: permeability; damage; repair; inflammation

EVIDENCE OF AN ABNORMAL AIRWAY EPITHELIUM IN ASTHMA

Asthma is functionally characterized by airway hyperresponsiveness and variable airway narrowing, which are associated with airway inflammation and remodeling. Airway remodeling collectively refers to structural changes of the airway wall, which may include epithelial fragility (1, 2), goblet cell metaplasia (3), increased airway smooth muscle mass (4), and basement membrane thickening due to extracellular matrix deposition (5).

The airway epithelium is the site of first contact to inhaled aeroallergens and other noxious stimuli, and, as such, it is well equipped structurally and immunologically to deal with this burden (6). In particular, mucociliary clearance, controlled permeability through adhesion junctions, and regulation and integration of innate and adaptive immune responses are the

CLINICAL RELEVANCE

To date, modeling asthma in mice has largely focused on immune-mediated mechanisms and phenotype changes to mesenchymal cells and the products that they elaborate. However, there is an underappreciated opportunity to use these models to study the impact of structural and functional changes to the airway epithelium and translate these results back to human disease. This Review summarizes the status of models used to study airway epithelium biology in mice, the status of modeling asthma in mice, and how these models have been used to investigate airway epithelium biology, and suggests future avenues to improve existing models.

primary mechanisms by which the epithelium responds to maintain homeostasis in the airways (6). In individuals with asthma, compelling evidence is emerging highlighting a dysfunctional epithelium displaying abnormal structure, biochemical, and immunological profiles that collectively result in a compromised airway epithelial barrier.

Seminal studies by Dunnill and colleagues (1), Naylor (2), and Laitinen and colleagues (7) provided evidence that the airway epithelium undergoes structural changes highlighted by regions of airway denudation. More recent reports describe changes in expression of proteins integral to the structure of airway epithelial cells in individuals with asthma, such as reduced expression of tight junctional protein, zonula occludens-1, adherens junction protein, E-cadherin, and increased expression of the basal cell marker, cytokeratin-5 (8–10). Collectively, these data associate the damage/fragility of airway epithelium and altered expression of epithelial-specific structural proteins to an asthma phenotype, although a causal link to disease pathogenesis remains to be demonstrated. Changes in airway epithelium structure in individuals with asthma have been studied to a limited extent using *in vivo* mechanistic approaches with mouse models. Mouse model systems allow for easy assessment of airway epithelium structure and protein expression simultaneously throughout multiple airway generations using histologic approaches, which can be complemented by detailed ultrastructural analysis. For these reasons, we feel that *in vivo* mouse experiments are well suited to explore further the structural profiles of the airway epithelium, both in normal and in disease models.

The structural changes in asthmatic airway epithelium have recently been associated with increased epithelial permeability in functional studies examining technetium-labeled diethylenetriamine pentaacetate (Tc-DTPA) clearance (11). In patients with chronic persistent asthma, inhaled Tc-DTPA displays a shorter half-life in the lungs, suggesting increased permeability as com-

(Received in original form April 5, 2010 and in final form June 9, 2010)

This work was supported by funds from the Canadian Institutes of Health Research and the Johnson & Johnson Focused Funding program.

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Am J Respir Cell Mol Biol Vol 44, pp 431–438, 2011

Originally Published in Press as DOI: 10.1165/rmb.2010-0146TR on August 19, 2010

Internet address: www.atsjournals.org

pared with control individuals without asthma. However, it is difficult to explore the mechanisms underlying this observation in humans. A strength of using mice is that it is ethically possible to model acute exacerbations, induce selective airway epithelial cell damage, and/or use animals with genetic modifications to genes involved in epithelial function (e.g., E-cadherin) and subsequently monitor barrier function, airway inflammation, remodeling, and their impact on pulmonary physiology.

In addition to structural and functional differences between individuals with and those without asthma, epithelial cells from individuals with asthma display abnormal growth factor release and extracellular matrix production (12, 13), and this is associated with defective repair. These data suggest that, even in the absence of inflammation, asthmatic airway epithelial cells may have an intrinsically altered function. Airway epithelial expression patterns of epidermal growth factor (14), matrilysin (15), and/or keratinocyte growth factor (16) are also altered in asthma. *In vivo* mouse models are well suited to mechanistic probing of these and other mediators in airway epithelium biology in an intact physiological system.

Lastly, the airway epithelium is emerging as a crucial player in integrating innate and adaptive immune responses (17–20). The expression of pathogen recognition receptors, production of antimicrobial and antiviral mediators, chemokines capable of recruiting antigen-presenting cells, and mediators that may facilitate allergic sensitization clearly indicates that this tissue is more than a physical barrier preventing unwanted access to the lung compartment. Airway epithelium from individuals with asthma demonstrates elevated cytokines, thymic stromal lymphopoietin (TSLP) (21) and IL-33 (22), chemokines, IL-8 (23) and CCL-17 (24) (thymus- and activation-regulated chemokine), and other mediators (17). The exaggerated production of these cytokines and chemokines by the airway epithelium has been linked to dendritic cell activation (25) and robust allergic inflammation that likely contributes to asthma pathogenesis. To compliment the excellent reviews in this area that have focused on *in vitro* and clinical biopsy studies (17–20), we discuss relevant mouse models of allergen exposure that have focused on the immune mediator secretory capacity of the airway epithelium. Recent studies demonstrate that the airway epithelium is a crucial player in orchestrating immune responses, and that mouse models will continue to be a valuable tool to investigate these processes.

Except for the few studies discussed subsequently here, modeling asthma in mice has generally neglected the structure, function, and inflammatory role of the airway epithelium. In this Review, we address the following two questions: (1) what *in vivo* models and methods have been used to inform us about airway epithelium damage, repair, and immune responses? (2) How can we improve on existing mouse models to understand changes in airway epithelium biology in asthma? By way of answering these questions, we discuss the role that *in vivo* models have played in allowing investigators to understand the biological consequences of normal and abnormal airway epithelium. We highlight past efforts of modeling asthma in mice that have focused on the airway epithelium, and how future studies may be improved to accommodate the current clinical evidence of altered epithelium in asthma (19).

WHAT MOUSE MODELS HAVE TAUGHT US ABOUT AIRWAY EPITHELIUM DAMAGE, REPAIR, AND IMMUNE RESPONSES

Materials and Methods

The airway epithelium can be examined in small animal models using a variety of methods, including electron microscopy,

conventional histology, immunohistochemistry, apoptosis/proliferation assays, laser capture microdissection, and analysis of bronchoalveolar lavage (BAL) fluid. Matrilysin (matrix metalloproteinase-7) (15), markers of Clara cell maturation (26), and/or keratinocyte growth factor (16) may be particularly useful for investigating the temporality of airway epithelial repair processes, as these markers change rapidly after damage, with the latter improving airway epithelium permeability in an *in vivo* model of allergen exposure (27). Airway epithelium-derived immune responses can be directly assessed with specific immunohistochemical/fluorescence staining methods or indirectly measured by soluble mediator analysis of BAL supernatant.

In cases of airway epithelial damage and/or compromised repair, it is likely that greater epithelium permeability exists. In small animal models, protein content in BAL fluid has been used as a surrogate marker of permeability changes of the epithelium (28), although a limitation of this as an outcome is that it cannot be directly linked to epithelium permeability *per se*, as it will be influenced by changes in vascular permeability. Alternatively, monitoring the distribution of intratracheally instilled tracer molecules may provide a method for repeated measurement of airway epithelium permeability (29). We have recently developed a mouse model of Tc-DTPA imaging using single-photon emission computed tomography that allows us to determine the lung clearance of tracer over time as a functional measurement and indirect marker of airway permeability, as has been done in humans (11). A limitation of this technique is that it is unable to discriminate between changes in airway or alveolar epithelial permeability. The static distribution of tracer molecules to assess increases in permeability can be performed using ultrastructural methods, as has been performed in guinea pigs with horseradish peroxidase (30). In these studies, allergic airway inflammation was associated with increased deposition of horseradish peroxidase between airway epithelial cells, which was suggestive of increased permeability.

For reference, we provide an annotated table highlighting specific mouse studies that contain methods for detecting biological parameters relevant to airway epithelium damage/repair, cell cycle, and barrier function (Table 1). From a pathological perspective, we propose that the term “epithelial damage” should be reserved for studies that observe and report both structural (e.g., cell organization) and functional (e.g., barrier function) changes that deviate from the normal phenotype. From this definition of “epithelial damage,” we propose that the term “phenotype change” be ascribed to studies where changes in immune profile, protein/RNA expression, and/or cell cycle are reported without structure and function data. In the future, adapting additional methodologies from human/non-mouse animal models focusing on the airway epithelium (30, 33), and mouse studies examining epithelium in different organs (34), may provide even more insight.

Allergic Models

Mouse models of allergen exposure designed to mimic asthma have been used extensively to study airway inflammation, remodeling, and airway hyperresponsiveness (35). Apart from reviews that have focused on goblet cell metaplasia (36), the epithelium has received comparatively little attention. As such, it is difficult to ascertain whether the current mouse models are useful or not in understanding the contributions of abnormalities of airway epithelium structure and function to the disease process. We emphasize that future studies using mouse models of allergen exposure to mimic asthma should incorporate outcome measurements that monitor airway epithelium damage/repair, immune profile, and/or function. In doing so, we will gain insight into the

TABLE 1. AIRWAY EPITHELIAL STRUCTURE/FUNCTION METHODOLOGIES CURRENTLY USED IN MOUSE MODELS

<i>In vivo</i> Biological Parameter Measured	Category of Method	Outcome Measurement	References	
Epithelial structure—damage/denudation/repair	Electron microscopy—TEM	Ultrastructure	(75–77)	
	Electron microscopy—SEM	Ultrastructure	(45)	
	Histology	H&E staining	(28)	
		KGFR	(16)	
		Markers of Clara cell maturation	(26, 78)	
		EGF/EGFR	(79)	
		Gene expression (laser capture of epithelium)	TGF-β1	(39)
	Cell proliferation	Gene expression (whole lung)	KGf mRNA	(31)
		BAL cell counts	Epithelial cells	(28, 48)
		Histology	Ki67	(80)
PCNA			(16, 31, 81, 82)	
BrdU			(28, 79)	
Cell death—necrosis and apoptosis	Electron microscopy—TEM		Ultrastructure	(45, 77, 78)
	Electron microscopy—SEM	Ultrastructure	(45, 77, 78)	
	Histology	TUNEL	(37, 81–83)	
		Caspase-3 activation	(16, 37, 84)	
		Apoptosis associated cleavage products	(37, 84)	
		Uptake of ethidium homodimer	(83)	
		bax, bcl-x, Fas	(32)	
Barrier function	Clearance of instilled reagent	Tc-DTPA	(85)	
	Electron microscopy—TEM	Ultrastructure	(30)	
	Histology	Tight junction proteins	(59)	
	ELISA on bronchoalveolar lavage samples	BAL protein content	(28)	

Definition of abbreviations: BAL, bronchoalveolar lavage; BrdU, bromodeoxyuridine; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; H&E, hematoxylin and eosin; KGF, keratinocyte growth factor; PCNA, proliferating cell nuclear antigen; SEM, scanning electron microscope; Tc-DTPA, technetium-labeled diethylenetriamine penta-acetate; TEM, transmission electron microscope; TGF- β 1, transforming growth factor- β 1; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

relevance that existing mouse models of allergen exposure have in modeling airway epithelium changes observed in human subjects. Subsequently, we highlight the few studies that have investigated airway epithelium—focused outcomes.

Ovalbumin (OVA) exposure remains a common method used to model asthma in mice. Dorscheid and colleagues (37) used an OVA exposure model to examine the role that corticosteroids may play in airway epithelial apoptosis and damage. In this study, epithelial cells were counted in BAL fluid and used as a surrogate marker for epithelial shedding. OVA exposure induced epithelial shedding and apoptosis, and both of these processes were insensitive to steroid treatment. The data support the notion that allergen exposure may contribute to airway epithelial damage, and that conventional therapies are unable to prevent this process.

Varying the duration of models of allergen exposure can elicit either acute or chronic changes in airway inflammation and structure (38). In contrast to the acute OVA exposure protocol used by Dorscheid and colleagues (37), Kelly and colleagues (39) used a repeated chronic OVA exposure over 12 weeks and identified key fibrotic mediators that were elevated in the airway epithelium by using laser capture microdissection methods. Chronic allergen exposure resulted in elevated TGF- β 1 and plasminogen activating inhibitor-1 mRNA in airway epithelium that was associated with increased collagen deposition in the airway wall. These results demonstrate that mice are capable of modeling allergen-induced changes to gene expression and structure within the epithelial mesenchymal trophic unit.

Recent exciting studies by Bates and colleagues (40, 41), who developed a mouse model of allergen exposure with increased airway sensitivity to nebulized methacholine, have explored the use of cationic proteins, specifically poly-L-lysine (PLL). Bates and colleagues hypothesized that disruption of tight junctions between airway epithelial cells by PLL would compromise the airway epithelial barrier and provide greater access of inhaled methacholine to the subepithelial regions, resulting in increased airway sensitivity. The instillation of PLL alone or in conjunc-

tion with OVA produced marked increases in airway sensitivity (40, 41). These data support the hypothesis that cationic proteins, such as those that could be released by eosinophils, may alter airway epithelial permeability. More significantly, the data show that compromising the epithelial barrier itself is sufficient to induce airway hyperresponsiveness.

Allergen exposure studies are beginning to highlight the importance of the immune secretory capacity of the airway epithelium. Zhou and colleagues (25) have explored the role of TSLP in mice after OVA exposure. TSLP is highly expressed in airway epithelium, and is capable of activating resident dendritic and mast cells, resulting in a T helper cell type 2 inflammatory phenotype (42). Allergen exposure to wild-type and TSLP receptor-deficient mice in conjunction with parallel studies with transgenic mice overexpressing TSLP in airway epithelium were used to demonstrate that TSLP was sufficient and necessary for the development of an allergic phenotype. Additional experiments by Weckmann and colleagues (43) further implicate the secretory role of the airway epithelium in response to allergen exposure. Using wild-type and TNF-related apoptosis-inducing ligand (TRAIL)-deficient mice, combined with short interfering RNA methods, TRAIL in the airway epithelium of mice was implicated in development of a T helper cell type 2 inflammatory phenotype similar to that observed in human asthma (43). Collectively, these studies support putting the airway epithelium immune secretory role at the forefront for development of pathology and dysfunction associated with the asthma phenotype (17, 19). In addition, they emphasize the clinical relevance and increasing requirement for examination of airway epithelium processes in models of allergen exposure in mice.

Environmental/Chemical Exposure Models

Naphthalene exposure by intraperitoneal injection routes results in selective Clara cell damage due to cytochrome P450 produced reactive cytotoxic metabolites (44). Naphthalene is a common component of cigarette smoke, diesel exhaust, and

pesticide formulations, and may be relevant to several human respiratory conditions (45). Ultrastructural and histologic analysis demonstrates naphthalene-induced Clara cell vacuolation with associated epithelial cell shedding in the airway lumen. These effects are observed 24 hours after exposure, and persist until at least 10 days, with resolution and epithelial reconstitution complete by 20 days. Unlike mechanical wounding models that disrupt the proximal airways, naphthalene exposure results in damage to the more distal bronchiolar regions of the airway tree. These models continue to be useful in monitoring the spatial and temporal expression of airway epithelial cell markers during repair, and can also be used to understand the contribution of specific cell types to remodeling (46).

The direct, selective targeting of airway epithelial cells by ozone is unclear, but has been explored in mice in the context of mast cells (28). In humans, ozone exposure results in airway hyperresponsiveness, although the precise mechanisms and any effects on airway epithelium are unknown (47). Longphre and colleagues (28) exposed wild-type and mast cell-deficient mice to either air or ozone, and examined the responses of airway epithelium. In wild-type, but not mast cell-deficient mice, ozone induced epithelial proliferation, as assessed by bromodeoxyuridine incorporation, as well as "focal areas of epithelial necrosis and exfoliation" in the conducting airways. The results suggest that oxidative stress may lead to airway epithelial damage and repair in a mast cell-dependent fashion. Future studies using these murine models may be useful in dissecting out the relationship between the epithelium and recruited inflammatory cells.

Martin and colleagues (48, 49) have developed and characterized a mouse model of chlorine gas exposure that results in airway hyperresponsiveness and substantial airway epithelial damage after a single 5-minute exposure. In humans, accidental exposure to chlorine gas can induce airway hyperresponsiveness. In the mouse experiments, a dose-response effect to chlorine exposure is observed for the number of airway epithelial cells recovered in BAL fluid. Airway epithelial cell desquamation and apoptosis was observed in the acute injury period in less than 24 hours after exposure. Initial airway epithelial cell damage was followed by proliferation that resulted in a repaired airway epithelium by 10 days after exposure. Of particular relevance to an asthma phenotype, the studies by Martin and colleagues demonstrate that damage to the airway epithelium can be accompanied by functional changes in airway physiology, such as airway hyperresponsiveness.

Genetic

The use of transgenic mice to examine airway epithelium has exploited the restricted expression of the Clara cell secretory protein (CCSP) to ablate selectively Clara cells from mouse airways (50). A herpes simplex virus thymidine kinase suicide gene was expressed within all CCSP-positive cells in the airway epithelium of mice. Subsequent ganciclovir exposure resulted in the selective ablation of the CCSP-positive cells in these transgenic mice. The transgenic mice generated demonstrate a phenotype of aberrant epithelial repair that does not result in complete reconstitution of the epithelium (50). An additional model of defective epithelial repair involves the use of matrilysin-deficient mice (15). Both of these mouse strains may be well suited to examine the functional consequences of aberrant/impaired airway epithelium repair.

Mechanical Models

Although the primary emphasis of this Review is on mouse models, we must discuss the pioneering work by Erjefalt and colleagues (33, 51), who assessed airway epithelial damage and

repair processes in guinea pigs. In these studies, a longitudinal strip of epithelium denudation 800- μ m wide was created without damaging the basement membrane or causing bleeding. In this model, indices of wound repair were seen as early as 15 minutes after damage. First, epithelial cells adjacent to areas of wounding undergo rapid dedifferentiation, characterized by a flattened migratory phenotype that covers the wound within 8 hours. Differentiation of the newly migrated epithelial cells into a mature barrier with tight junctions occurs by 5 days. Interestingly, using these models, Erjefalt and colleagues (33) provided early insight into the role that the airway epithelium may play in induction of airway inflammation and subsequent development of pathology in individuals with asthma. Models of mechanical wounding have the strength that they can be performed on naive animals or animals subjected to other protocols (i.e., allergen exposure) and/or treatments (i.e., corticosteroids). To our knowledge, mechanical wounding models have not been reported on mice to date. We believe that these mechanical models would be useful in expanding on previous results from *in vitro* experiments, which demonstrated production of growth factors that can act on mesenchymal cells in response to mechanical damage (52).

HOW CAN WE IMPROVE ON EXISTING MOUSE MODELS TO UNDERSTAND CHANGES IN AIRWAY EPITHELIUM BIOLOGY IN ASTHMA?

As mentioned previously here, a systematic analysis of airway epithelium damage/repair, barrier function, and immune secretory capacities has not been performed in studies modeling asthma in mice. Historically, modeling asthma in mice has explored the immune components of the disease, with minimal attention paid to any contributions made by the airway epithelium. However, with emerging evidence suggesting that immune responses are downstream of initiating events that occur at the airway epithelium-environment interface (6), it is prudent to improve our existing mouse models to effectively determine the role of the epithelium in asthma. First and foremost, existing models should incorporate analysis of the airway epithelium for damage/repair, barrier function, and immune secretory capacity. In line with this, mouse models have recently been used to characterize the role that airway epithelium-derived TSLP (25) and TRAIL (43) play in development of asthma. Mouse models can be taken further to study interactions between the airway epithelium and local dendritic cells (20), release of cytokines and chemokines that influence T-cell populations (17), identification of factors that promote B-cell activation (53), and other airway epithelium/immune cell interactions currently unexplored.

Moving toward more clinically relevant (inhaled) allergens is also a necessary step. Existing mouse models of allergen exposure routinely use single allergens, and may be improved by addition of other allergens (e.g., house dust mite [HDM] plus OVA). The addition of viral, bacterial, air pollutant, oxidant, or other clinically relevant substance to current mouse models of allergen exposure may also more accurately produce changes in airway epithelium biology observed in individuals with asthma. Initial complex exposure models are being developed, with recent work by Hollingsworth and colleagues (54) exploring combined allergen and ozone exposure. The authors were able to demonstrate that ozone-induced dendritic cell activation and development of an allergic phenotype depended on Toll-like receptor-4. Likely playing a role in facilitated allergic sensitization and dendritic cell activation in this model is the airway epithelium (20), although this remains to be explored.

With the introduction of widespread corticosteroid treatment for patients with asthma, many currently reported asthma exacerbations are noneosinophilic, and are thought to be secondary to

airway infection (55). Thus, the interest in the interaction between pathogens and mouse models of allergen exposure is increasing, but much remains unknown, including the effects of dual pathologic processes on the airway epithelium (56). In a complex exposure model exploring this area, Bartlett and colleagues (57) have developed a mouse model of rhinovirus-induced exacerbation of an existing allergic airway phenotype. On the background of OVA-induced allergic airway inflammation in BALB/c mice, rhinovirus-1B induced greater airway inflammation and dysfunction as a result of replication in the airway epithelium. Rhinovirus-1B disrupts airway epithelium tight junctions in mice by reorganization of zonula occludens-1 expression in airway epithelial cells, and may contribute to altered airway epithelium permeability *in vivo* (58, 59), although this awaits confirmation. It will be exciting to determine the role that the airway epithelium plays in models of virus-induced allergic airway exacerbations, and how any changes in airway permeability alter airway function. Staphylococcal α toxin has been demonstrated to cause increases in mouse tracheal permeability in an *in vitro* assay, although this has not been corroborated *in vivo* (60). *In vitro*, histologic analysis of the staphylococcal α toxin-infected tracheas demonstrated selective loss of airway epithelial cells, with a rapid change in airway permeability observed within 15 minutes. The rapid onset of changes in airway permeability associated with epithelial loss could make this a useful *in vivo* model for observing the early response to airway epithelial damage. The combination of virus/bacteria and allergic inflammation models may induce changes in airway epithelium biology that are more consistent with exacerbations in the human population, and should be pursued.

A potentially relevant mechanism of airway epithelium damage in mice may be the actions of proinflammatory mediators released from eosinophils in response to allergen exposure (61). To date, eosinophil degranulation in mice has been a questionable occurrence, and only recently has this been explored thoroughly with electron microscopy (62). Clear demonstration using electron microscopy techniques shows that, in response to OVA exposure, wild-type C57Bl/6 mice have lung eosinophils that do not undergo degranulation. In contrast, Ochkur and colleagues (62) used IL-5/eotaxin-2 dual transgenic mice that do show evidence of degranulation. Ochkur and colleagues did not discuss any changes in airway epithelium structure or permeability, although their airway function data support the concept of a compromised epithelial barrier function. Mouse models of allergen exposure that incorporate eosinophil degranulation, such as those using the transgenic mice generated by Ochkur and colleagues, may improve existing models, and will prove to be useful in determining how these cells alter airway epithelium biology. Additional models incorporating known changes in airway permeability, such as those described by Bates and colleagues (40, 41), will also be invaluable.

HDM extract has been demonstrated to be proteolytic and involved in cleaving occludin in tight junctions of airway epithelial cells *in vitro* (63, 64). Supporting *in vivo* data investigating the protease activity of HDM extract have not been published. Demonstration of *in vivo* proteolytic activity of HDM on epithelial junctions would support the use of an HDM model allergen exposure to investigate increased permeability in airway epithelium. If the *in vitro* proteolytic activity of HDM extract is conserved *in vivo*, airway epithelium tight junctions should be cleaved and be apparent by standard histologic and/or ultrastructural techniques. Alternatively, assays using intranasally instilled tracer molecules after exposure with extracts, such as HDM, would be beneficial in determining the effects on tight junctions and epithelial permeability. Additional allergens with or without proteolytic activity may

also be explored in the context of airway epithelium damage and repair (65, 66).

Based on *in vitro* studies, both cysteine and serine proteases can act to cleave airway epithelial tight junctions (63, 64). Papan is a cysteine protease that has a conserved protein sequence, active site, and three-dimensional structure similar to the HDM-derived protease, Derp1 (67). The application of papain in a mouse model of allergen exposure has been explored (67, 68). Although the airway epithelium was not focused on in these studies, it is important to determine the effect of protease exposure on airway epithelium structure and function in the future.

Review of the literature describing mouse models of airway epithelium damage demonstrates a potential theme of oxidative stress in the airways. Oxidative stress may be relevant in asthma, and is not directly incorporated into classical mouse models (69). Incorporation of oxidative stress into a mouse model with pre-existing allergen-induced airway inflammation may prove useful in establishing models that more closely resemble the human condition.

We have reported increased expression of p63 in asthmatic airway epithelial cells and side population cells, although the mechanistic link of these cells to asthma pathology remains to be determined (8, 70). In p63 knockout mice, an absence of basal cells is observed, despite a fully differentiated ciliated airway epithelium (71), suggesting that more than one progenitor cell type is available to generate a proper epithelium. However, a recent report using p63 as a marker of basal cells suggests that basal cells are stem cells of the airway epithelium, and can contribute to epithelium repair after damage (72). Considering this, we hypothesize that the absence of stem/basal cells would severely compromise the capacity of the epithelium to mount a productive repair response to insult. Unfortunately, p63 knockout mice die at birth (73, 74), meaning that conditional transgenic methods may need to be applied to explore more extensively the role of this protein in airway epithelium biology. In the broader context, epithelial progenitor cells and their self-renewing progeny have yet to be explored in mouse models of airways disease. Future mouse models may incorporate analysis of these cells by monitoring the cell cycle and localization of p63-positive cells.

Lastly, early-life exposure of mice to virus, bacteria, air pollutant, or oxidants could be followed by adolescent exposure to allergen to determine the consequences of early-life airway epithelium insult on susceptibility to allergen sensitization and/or allergic airway inflammation.

CONCLUSIONS AND FUTURE DIRECTIONS

The airway epithelium is an important barrier in the defense against inhaled allergens, pollution, viruses, and bacteria, and, as such, is armed with immunological and structural tools to handle these various insults. The mechanical barrier, which is key to proper airway epithelial function, consists of multiple cell types, each with their own unique role in host defense. Working in synchrony with the mechanical barrier to maintain a defense against insults is the immune and secretory capacity of the airway epithelium. Perturbations in the harmony of epithelial function may promote a microenvironment favoring unwanted responses of allergic sensitization, inflammation, and airway remodeling, suggesting that airway disorders, such as asthma, must involve the epithelium. We suggest that mouse models are suitable to test exciting hypotheses that focus on the airway epithelium as a major contributor to the asthma phenotype.

To investigate airway epithelium structure and function with *in vivo* model systems, objective methodologies must be imple-

mented and interpreted appropriately. Use of the term airway epithelium “damage” should be reserved for studies that clearly demonstrate a phenotype different from naive tissue state, with supporting measurements of dysfunction. Data demonstrating changes in protein/RNA/histology that are presented without structural/functional outcomes should be labeled as “phenotype” changes rather than “damage.” Future studies should incorporate histological or ultrastructural techniques, functional measurements of permeability and immune function, and expression of proteins/genes to provide comprehensive insight into airway epithelial status in models of asthma.

Mouse models of allergen exposure to date have largely focused on immune components of the disease, extracellular matrix deposition, and smooth muscle accumulation. A shift in focus to the role of the airway epithelium regulating several key functional and immunological abnormalities in asthma will require investigators to begin exploring this tissue in asthma models (40, 41). Focusing on the airway epithelium in existing and future mouse models of airways disease may unveil pathways integral to aberrant epithelium structure and function. Within these pathways that govern airway epithelium biology, there likely awaits exciting new therapeutic targets for treatment of airway diseases, such as asthma.

Author Disclosure: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgments: J.A.H. is a recipient of a Canadian Institutes of Health Research (CIHR) Integrated and Mentored Pulmonary and Cardiovascular Training Trainee Fellowship (IMPACT). T.-L.H. is a recipient of a CIHR/Canadian Lung Association/GSK, CIHR IMPACT and Michael Smith Foundation for Health Research Trainee Fellowships. D.A.K. is the Canada Research Chair in Airway Disease and Michael Smith Foundation Career Investigator.

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